Mississippi Canyon 252 Deepwater Horizon Oil Spill

NRDA Sampling Plan

MESOPHOTIC REEF FOLLOW-UP CRUISE PLAN

Deepwater Benthic Communities Technical Working Group

Final: 1 August 2012

Approval of this work plan is for the purposes of obtaining data for the Natural Resources Damage Assessment. Each Party reserves the right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

The Trustees have developed a preliminary conceptual model of the DWH release, potential pathways and routes of exposure, and potential receptors. This preliminary model has informed the Trustees' decision to pursue the studies outlined in the work plan. By signing this work plan and agreeing to fund the work outlined, BP is not endorsing the model articulated in the work plan.

APPROVED:

Robert W. Ricker

Department of Commerce Trustee Representative:

August 24, 2012

Date

State of Louisiana Representative:

9/20/12

Date

Joyce Miley

BP Representative:

Aug 28, 2012

Date

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Deepwater Benthic Communities Technical Working Group

Final: 1 August 2012

CRUISE DATES: 15 September-1 October 2011

PREPARED BY: Dr. Kenneth J. Sulak, USGS, fish ecologist, USGS-SESC, Gainesville, FL, ksulak@usgs.gov, 352-264-3500

EXECUTIVE SUMMARY: A post-spill mission was conducted in August 2010 aboard the NOAA RV 'Nancy Foster' to deep, shelf-edge, mesophotic reefs located north and east of the Macondo wellhead site. The two target study (test) reefs, potentially within the zone of influence of the oil spill, were Alabama Alps Reef and Roughtongue Reef, both large high-relief platform reefs within the Pinnacles reef tract, northeastern Gulf of Mexico (NEGOM). The reference reef, outside the zone of potential influence of the oil spill, was Coral Trees Reef (CT) located on the West Florida shelf edge. This was a compromise reference reef for 2010. The originally-preferred reference reef, Madison-Swanson South Ridge (MSSR), could not be studied in 2010 (during the Mesophotic Reef Leg II of the Tier 1 coral assessment cruise on the Nancy Foster) due to a reduction in science sea days to accommodate the needs of Leg I of the study plan, which evaluated the deeper cold water coral and seep communities (Deep Benthic). Multiple objectives were pursued during the 2010 Mesophotic Reef mission, as per the original 2010 study plan. However, the primary objective was to conduct standardized quantitative ROV video transects on both the test and reference reefs to statistically compare abundance and diversity of the reef fish community, with particular emphasis on planktivore reef-fish species. Preliminary Trustee data analyses showed statistically significant lower numbers of total fishes, planktivore reef fishes, and large perciform predator fishes on the two test reefs as compared to the reference reef[1]. No trenchant pattern in diversity was evident, given the sample sizes available for analysis. The fundamental goal of the proposed 2011 follow-up mission is to re-evaluate reef-fish abundance and diversity, one-year out, on the same two Pinnacles tract test reefs, in comparison with MSSR and CT West Florida shelf reference reefs. Ancillary objectives regarding sediment hydrocarbon determination (sampling via tube coring), coral status (visual observation, imagery, and tissue sampling), and above-reef water column parameters (CTD profiles) will be the same as undertaken in 2010. The Semi-Permeable Membrane Device (SPMD) component from 2010 will not be repeated during the proposed 2011 mission.

[1] Result is based on preliminary analysis of available data. BP's approval of this work plan is not an admission of the accuracy of any data interpretations or analyses relied upon or referred to herein.

INVESTIGATION LEADERS[2]:
Dr. Kenneth J. Sulak, USGS, fish ecologist, USGS-SESC, Gainesville, FL, ksulak@usgs.gov, 352-264-3500 (chief scientist and day-shift scientific party chief)
Dr. Amanda Demopoulos, USGS, benthic ecologist, coordinating sediment collections for hydrocarbon analysis, USGS-SESC, Gainesville, FL, ademopoulos@usgs.gov, 352-264-3490

KEY PERSONNEL[3]:
Dr. Ian MacDonald, Florida State University, marine scientist
Dr. Scott France, University of Louisiana-Lafayette, coral biologist, post-cruise, in-laboratory coral identification operations
Dr. Peter Etnoyer, NOAA, coral scientist
Michael Randall, USGS, fish biologist, night shift scientific party chief
Ann Foster, USGS, GIS expert & WinFrog navigation
Jenny Adler, Jacobs Contractor, research assistant to Kenneth Sulak, reef-fish identification and GIS assistant

I: BACKGROUND: Dramatic high-relief (10-15 m) fossil calcareous reefs (known as "The Pinnacles", e.g., "Alabama Alps Reef", Figs. 1, 2) occupy the shelf-edge in the eastern end of an area of dense oil development (Fig. 3) in the Gulf of Mexico (GOM) off Louisiana and Mississippi. Such shelf-edge hard-bottom features, have been described as 'Underwater Oases' (NOAA Ocean Explorer website, Islands in the Stream 2001-2002: "Exploring Underwater Oases Expedition": http://oceanexplorer.noaa.gov/explorations/02sab/ background/edu/education.htm), and are nodes of elevated biodiversity due to topographic-hydrographic-faunal interaction. They support GOM snapper-grouper, amberjack and porgy fisheries. USGS high-resolution multibeam mapping of the Pinnacles and comparative hard-bottom features off the West Florida Shelf (Gardner et al. 2000, 2002, 2003) has shown that the area of influence of such features extends beyond the limits of the actual hard-substrate reefs. Acoustic backscatter profiling (Fig. 2) shows that weathered calcareous reef hash surrounding reef complexes can extend out for miles, creating an apron of coarse texture calcareous sediment, a second type of habitat (versus the general fine-grained clastic shelf sediment regime) supporting soft-bottom benthic communities. This extended zone of reef influence has been described as ecologically important since many site-attached reef fishes, other than planktivores, forage on this reef-hash apron (Weaver et al. 2002).

Although a NOAA study on the continental shelf in August 2010 found no indication of concentrations of MC252 oil (in surficial sediments) above method detection limits, and concluded that total hydrocarbon concentrations were low compared to known impacted areas (Cooksey et al. 2010), according to NOAA models[4] the Louisiana-Mississippi shelf-edge mesophotic Pinnacles reefs lay immediately beneath the zone of maximum potential surface spread of oil from the Deepwater Horizon (DWH) oil spill (Figs. 3 and 4) for an extended period of time. A composite map of the extent of surface oil spread, prepared via ArcGIS software

[2] Dr. Demopoulos will not be participating in field activities, but will be coordinating sediment collections for hydrocarbon analysis, assisting with post-cruise data analysis and reporting, and will be sending two assistants on the cruise.
[3] Dr. Scott France and Peter Etnoyer will not be participating in field activities, but will be participating in post-cruise data evaluation, and will be sending assistants on the cruise.
[4] BP's approval of this work plan is not an admission of the accuracy of any models relied upon or referred to herein, including, but not limited to, the modeling or interpretations included in Figures 3 and 4, and also is not an admission of the validity or feasibility of any hypothesized exposure or impact pathway relied upon or referred to herein.

from periodic NOAA online reports during April-July 2010, is presented for reference in Fig. 3. Also presented for reference in this regard is Fig. 4, a commercially-available satellite-imagery interpretation of the spread of surface oil as of 21 May 2010 (www.roffs.com). The deep reef ecosystem is fueled by surface-derived plankton (Sulak et al. 2008a). The 'mesophotic' qualifier means that the reefs lie at depths (60-90 m) where sunlight still penetrates, but not sufficiently to support photosynthesis. Site-attached planktivorous fishes, sessile reef invertebrates (corals, sponges, and other filter-feeders), and the macrofaunal benthos depend directly (vertical transport of particulates) and indirectly (horizontal transfer through the reef food chain) upon the fallout of surface-derived carbon. In turn, the dominant reef predators depend on the small relatively abundant planktivorous reef fishes as their food source (Weaver et al. 2002), and upon the sessile invertebrates as shelter. The planktivorous fish are small-bodied, active, visual, diurnal predators with high metabolic needs but limited energy reserves. They depend on a daily supply of plankton to survive, grow and reproduce. Three potential deep-reef fish community impacts from surface oil may be hypothesized based either on food deprivation or exposure to hydrocarbons or other spill-related factors (e.g., dispersant; data are currently unavailable to test these hypotheses):

1) Lowered survival due to denial of surface-derived plankton resulting in poor condition.
2) Elevated predation vulnerability and mortality while attempting to feed off bottom under conditions of plankton scarcity and diminished illumination due to surface and suspended oil.
3) Elevated predation vulnerability and mortality due to hydrocarbon or dispersant toxicity resulting in abnormal behavior.

Shelf-edge reefs on the West Florida Shelf (WFS), such as MSSR, remained out of the zone of surface oil spread throughout the course of the Deepwater Horizon (DWH) event. Reefs like MSN and MSSR may be used as reference mesophotic study sites to compare fish abundances and epibenthic community health at the same time period, and may be used to place results from AAR and RTR into temporal context. Some previous research and faunal exploration (Weaver et al. 2002, NOAA Ocean Explorer 2001 Island in the Stream Expedition) appear to have documented a fish community of essentially uniform composition occupying the northern Gulf of Mexico fossil shelf-edge reefs at 60-90 m depth, east of the Mississippi River, Alabama Alps to Madison-Swanson area. Some stable carbon isotope studies suggest that the community influence of outflow of the Mississippi River is limited to the immediate nearshore zone, with no measurable contribution to or effects upon outer shelf ecosystems, including deep reefs (Thayer et al. 1983, Sulak et al. 2008a).

The plankton-based deep-reef fish community could potentially be vulnerable to oil or dispersant impacts due to death of the surface food base, and/or light extinction that might impede or prevent visual plankton-picking feeding. Live soft coral/sponge habitat is potentially vulnerable to petroleum hydrocarbons or dispersants, but may otherwise be more resilient since growth is slow (Andrews et al. 2002) and metabolic rates are very low and feeding can be discontinued during adverse conditions. Reef fishes and reef-apron fishes that feed on infaunal benthic invertebrates could potentially be impacted due to exposure to hydrocarbons and/or dispersants or indirect effects upon these benthic animals[4].

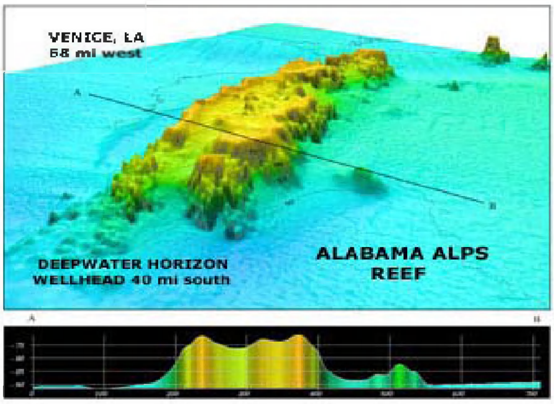


Fig. 1. Example of the high-relief physiography of a typical Pinnacle reef.

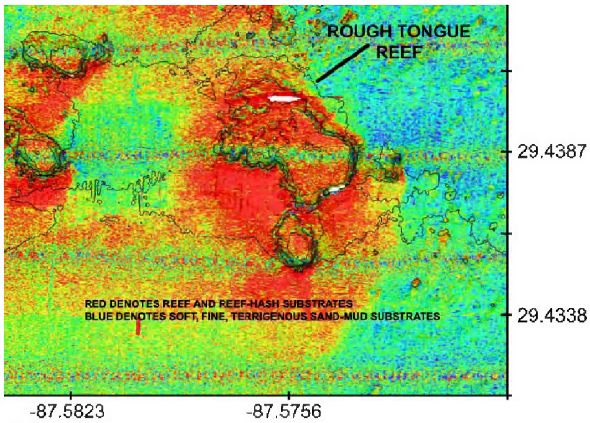
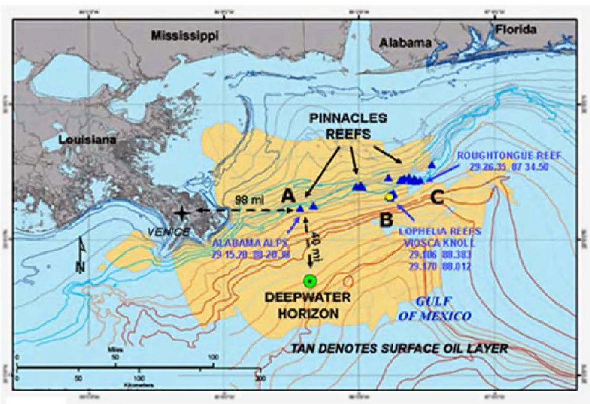


Fig. 2. Acoustic reflectivity revealing extended zone of unique reef-hash substrate (red off-reef areas).

II: HYPOTHESES: The central 2010 null hypothesis was that no statistically significant differences would be found in numbers of fishes enumerated per unit time in ROV video transect analyses conducted at the CT reference reef site compared to each of the two AAR and RTR test sites. This hypothesis appears to be falsified for 2010 ROV video transecting based on preliminary Trustee analysis of the 2010 data (data provided separately). That is, total fish numbers and planktivore numbers per unit time were statistically lower on the two test reef sites (those reefs potentially affected directly or indirectly by the oil spill), versus the reference reef site.



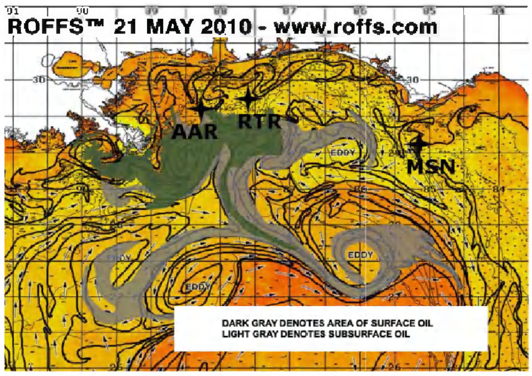


Fig. 3. Composite interpretation of NOAA imagery of surface oil extent May-July 2010.

Fig. 4. Roffs interpreted satellite imagery of surface and subsurface extent of oil as of 21 May 2010 (www.roffs.com).

Additionally, the abundance dominance order of fish species in the fauna differed at the test reef sites versus the reference reef site.

The central 2011 investigation null hypothesis remains the same as for 2010, but in 2011, both the more comparable in size, structure, and relief Madison-Swanson reef site and the CT site will be studied. In addition, fish abundance and diversity will be evaluated to determine if 2010 results reflected short-term temporal difference or spatial events potentially unrelated to the oil spill (if the null hypothesis is accepted in the current study).

III: OBJECTIVES:

1) Conduct ROV reef fish community video transect imaging (standardized nominal 5-min transects on reef-top biotope) at two test (potential oil impact) mesophotic Pinnacles reefs (AAR and RTR) and at two comparative reference WFS mesophotic reefs: MSSR and CT reef. Restriction of transects to reef-top biotope will insure comparability in reef to reef contrasts. A minimum total sample of 20 nominal 5-min transects per reef study site is the goal for each of the primary study reef sites. An accompanying goal is to accomplish an equal number of daytime versus nighttime transects at each study site, except CT reef (where only daytime ROV transects will be accomplished to provide precise comparability with 2011).
2) Accomplish quantitative analyses (Refer to Methods section below and Appendix A) for 2011 ROV video transects for fish quantification and statistical comparison with 2010 ROV transects on AAR and RTR test sites and CT reference site (using transects from the MSSR and CT as the comparative 2011 reference reef site).
3) Utilize high-quality 2011 *in situ* digital still ROV imagery (alternating with 5-min bottom transects) to document taxonomic identity and diversity (species richness) of megafaunal fish fauna for comparison with documented pre-2010/2011 diversity (Weaver et al. 2002). An Ethernet underwater digital still camera will be used to supplement the fixed-mount ROV digital still camera.
4) Utilize high-quality 2011 *in situ* video and digital still ROV imagery to document apparent health and condition of the sessile invertebrate fauna (with focus on soft corals – P. Etnoyer, collaborating PI), comparatively contrasting the two test and comparative reference sites. The Ethernet underwater digital still camera will also be used in this regard.
5) Use an autonomous bottom camera, deployed by ROV, to conduct 2-day *in situ* digital still sequence (interval firing, 10 images per hr) imagery on high quality reeftop habitat to document the diurnal/nocturnal pattern of variation in abundance and activity of the primary target reef fish. This will help ground truth ROV video quantification of these fish, and provide an independent check on ROV disruption of normal fish behavior.
6) Using the ROV manipulator, collect small branches or samples of living tissue from soft and hard corals and other dominant reef invertebrates to be preserved and used in definitive laboratory identifications at both the gross morphological and genetic levels (S. France, collaborating PI). Using the ROV suction samples, collect additional voucher invertebrates to document the sessile and sedentary fauna of both test and reference reefs. Tissue from soft and hard corals and other associated reef invertebrates (e.g., sponges, brittle stars) will be sampled from each site for hydrocarbon analyses.
7) Using core tubes deployed by the ROV manipulator, collect a minimum of ten sediment cores for hydrocarbon analyses from the soft sediment apron surrounding the reef platform at each primary study site. Cores will be obtained from ten separate locations representing all four compass quadrants around the reef platform at each study site. Hydrocarbon analyses of the sediments will be conducted according to the NOAA QAP (NOAA 2011) and will consist of methodologies consistent with *ROV Sediment and Bottomwater* (HOS Sweetwater 2/4/6) Surveys and the *Softbottom Sediment Survey* Programs sponsored by the Deepwater Benthic Communities Technical Working Group.

IV: METHODS FOR THE 2011 FOLLOW-UP ROV MISSION: Reef study sites and potential ROV dive locations are specified in Appendix B. ROV methods for 2011, based on those from 2010, with modification, are given in brief in Appendix C. Video analysis methods (in laboratory) are given in Appendix A. MSSR will be the primary 2011 West Florida shelf-edge reference site in 2011. CT reference reef will be revisited to provide continuity to the 2010 survey and as a comparison to MSSR. For this study plan, the Madison-Swanson reef complex, focusing in 2011 on MSSR dive locations (Appendix B), will serve as the primary reference reef site, based on physiography and areal extent similar to the AAR and RTR target study sites. MSSR has also been chosen for 2011 given the existence of high-quality multibeam imagery for this site. Only low resolution imagery is available for MSN, which will be reserved as a contingency site, should commercial or military vessels be occupying MSSR during the 2011 mission).

Planktivore Abundance and Fish Community Diversity (Species Richness): The apparent difference in fish abundance between the test reefs and the CT reference reef noted in preliminary Trustee ROV transect videotape analyses from 2010 needs to be re-evaluated one year out from the DWH oil spill. It is possible that 2010 results represented a transitory aberration, a diurnal activity pattern shift, or a period of absence unrelated to oil impacts. This second ROV quantitative transect mission in October 2011 is proposed to test 2010 mission indications of community change. Revisiting the CT reference site will assist in testing spatial/temporal community change.

Autonomous Bottom Camera Deployment: One or two (depending on availability) autonomous (battery powered and deployed on bottom for later recovery) bottom cameras on loan from Florida State University will be deployed at each primary reef study site (MSSR, RTR, AAR) for a period of 48 hr. At each study site, an initial 3 hr ROV recon dive will be launched first. This will be launched at a site previously identified as having well-developed reef habitat, undertake habitat recon, and then be retrieved. The two autonomous bottom cameras will be loaded onto the ROV and transported to the bottom, and set in place in a location providing 360° visibility. The camera and lights will fire at pre-determined intervals (ten images per hour) to capture undisturbed (no ROV motors, lights, bubbles, etc.) imagery of mobile megafauna (fishes and mobile invertebrates) over the entire diurnal/nocturnal cycle for two days. The cameras will be retrieved by ROV at the end of day-2 at each primary study site. Imagery will be downloaded to harddrive in the laboratory van on the OSV, and backed up onto SD card for data security.

Coral Health and Condition – Follow-Up: Visual assessment of coral condition will be accompanied by close-up photography of corals, and by tissue collections to test for tissue damage and hydrocarbon concentrations. Six marked stations (three with apparently unhealthy corals and three with apparently healthy corals) will be established using sea bottom markers at the Alabama Alps Reef and Roughtongue Reef, respectively. Detailed high-resolution images will be collected at each station. The plan will be to focus the final 1.0-1.5 days of ROV operations, at each primary site, upon specimen collection and sediment collection. Sampling will target the dominant sessile invertebrate taxa typical of shelf-edge deep reefs (e.g., *Swiftia* and *Hypnogorgia*). The same species/taxa will be targeted for sampling at each of the four major sites to facilitate among-site comparisons. The ROV manipulator will be used to cut branches from selected soft coral specimens for subsequent laboratory identification, layout photographic documentation aboard the OSV, and for hydrocarbon analyses. An attempt will be made to select samples of any apparently unhealthy corals and comparative healthy corals of the same dominant species. As many specimens will be collected as the ROV samplers, biobox storage, and time available permits per reef site. At a minimum, at least 10 individuals of each species will be collected at each major site (e.g., RTR, AAR, MSSR, and CT) for hydrocarbon analysis.

Sediment Cores: A minimum of ten sediment push cores will be collected from each primary study site (MSSR, RTR, and AAR) for hydrocarbon determinations in the laboratory. Sediment cores will be collected in close proximity to the invertebrates collected for hydrocarbon analysis, when sediment is present in the environment. If not present, then sediment cores will be collected opportunistically from each of the four compass quadrants (N, E, S, W) while the ROV recons around the periphery of the reef platform. When an area of thick sediment is encountered in an area generally free of reef rock and rubble, a sediment core will be collected using discrete push cores (6.35 cm internal diameter) capable of collecting sediment to a depth of approximately 30 cm. 10 sediment push cores will be mounted to the ROV sled during each deployment and analyzed according to the methods described below (and in Table 1).

Table 1. Preservation, container, volumes and holding time requirements for sediment analyses.

Parameter	Lab	Method	Sediment needed	Container	Preservation	Holding Time
Parent and Alkyl PAHs; Saturated Hydrocarbons; Petroleum Biomarkers	Alpha Analytical	EPA 8270D / 8015	50 ml	8 oz wide mouth glass	-20C°	1 year

Sediment cores will be collected at each of the sites for hydrocarbon analysis, extruded and divided into vertical sections (0-1 cm , 1-3 cm, 3-5 cm and 5-10 cm deep). Sediments will be placed into clean glass jars with Teflon lid liners, invertebrates will be placed in either clean glass jars or wrapped in solvent rinsed foil, stored onboard ship at -20 ^{o}C (see Table 1), and transported to NRDA/Trustees analytical chemistry contractor (Alpha Analytical Laboratory) for extraction and analysis of hydrocarbons using approved NRDA QAP chemistry analysis and laboratory methods. The following measurements will be included: total petroleum hydrocarbons (TPH), also known as total extractable hydrocarbons (TEH), PAHs including individual parent and alkyl homologues, saturated hydrocarbons including alkanes and isoprenoids; and oil finger-printing diagnostics. Analyses will follow the methods provided in the NOAA MC 252 Analytical Quality Assurance Plan/Version 2.2 (NOAA 2011). Core tube and sediment processing tool decontamination methods are provided in Appendix E.

Epifaunal Invertebrate Collections: Invertebrates will be obtained to validate identifications for video transects and digital still imagery, for hydrocarbon analyses, and for potential documentation of injury or death. Collection of invertebrate specimens will be conducted primarily on ROV dives specifically devoted to specimen and sediment collection (final 1.5 days per each primary study reef). This will be done so as to avoid conflict between ROV transect and imagery operations (requiring a view unobstructed by collecting gear) and ROV collecting operations (requiring a forward work tray loaded with collection and storage gear). Emphasis in selection of specimens will be on soft corals. Other invertebrates will be collected opportunistically as they are encountered. At least 10 individuals of each species will be collected at each primary reef site (e.g., RTR, AAR, MSSR) for hydrocarbon analysis. The same species/taxa will be targeted for sampling at each of these sites to facilitate among-site comparisons. Invertebrates will be collected using the ROV manipulator and/or suction sampler, and stored in either a multi-chambered biobox or in the numbered suction-sampler chambers. Upon landing of the ROV and emptying of the biobox and suction sampler chambers, each coral collected will be photographed with the ROV station number. For soft corals, a subsample of one branch will be excised and retained for definitive morphological and genetic identification and permanent curation at the University of Louisiana-Lafayette (ULL) coral laboratory. The remaining portion of the coral, and whole specimens of other invertebrates, will be placed in either clean glass jars or wrapped in solvent rinsed foil, stored onboard ship at -20 ^{o}C, and transported to NRDA/Trustees analytical chemistry contractor (Alpha Analytical Laboratory) for extraction and analysis of hydrocarbons using approved NRDA QAP chemistry analysis and laboratory methods (NOAA 2011). The following measurements will be included: total petroleum hydrocarbons (TPH), also known as total extractable hydrocarbons (TEH), PAHs including individual parent and alkyl homologues, saturated hydrocarbons including alkanes and isoprenoids; and oil finger-printing diagnostics. Analyses will follow the methods provided in the NOAA MC 252 Analytical Quality Assurance Plan/Version 2.2 (NOAA 2011).

All sample containers will be cleaned and sealed at the surface. The biobox and slurp chambers and tubing will be washed in Liquinox solution and rinsed with de-ionized water prior to each dive. Immediately prior to deployment of the ROV, they will be filled with clean seawater and capped to prevent contamination of any hydrocarbons in the water column or during transit through the water column or along the seafloor.

Coral Identification: Corals present at each site will be tentatively identified by morphology, habitat, and coloration during real-time video monitoring during ROV video transecting and still imagery. Definitive ULL laboratory identifications of corals from 2010, mated with 2010 layout images of each specimen, will facilitate 2011 identifications. Additionally, 2011 coral subsamples taken will subsequently be definitively identified by ULL laboratory based on gross and fine morphology and genetic determinations. Subsamples will be permanently curated at ULL as voucher specimens to document 2011 mission collections.

All sample containers will be cleaned and sealed at the surface. The biobox and slurp chambers and tubing will be washed in Liquinox solution and rinsed with de-ionized water prior to each dive. Immediately prior to deployment of the ROV, they will be filled with clean seawater and capped to prevent contamination of any hydrocarbons in the water column or during transit through the water column or along the seafloor.

Water Quality Sampling: The ROV will be equipped with a CTD collecting the following real time water-column data on each ROV dive: conductivity, temperature, and depth, and the instrument includes fluorometers (CDOM, Aquatracka), turbidity meter, and DO probe. The CTD will provide a continuous record of all key abiotic factors conventionally measured at sea, in addition to the water column biotic factor Chlorophyll A.

Cruise Track: The intended cruise track is shown below (Fig. 5). Reference coordinates are provided for each of the primary study sites. Distances from port of departure and initial study site, and between primary study sites are indicated in nautical miles.

DATA OPERATIONS:

All results will be made available to BP/Cardno ENTRIX within a reasonable time after completion of analysis.

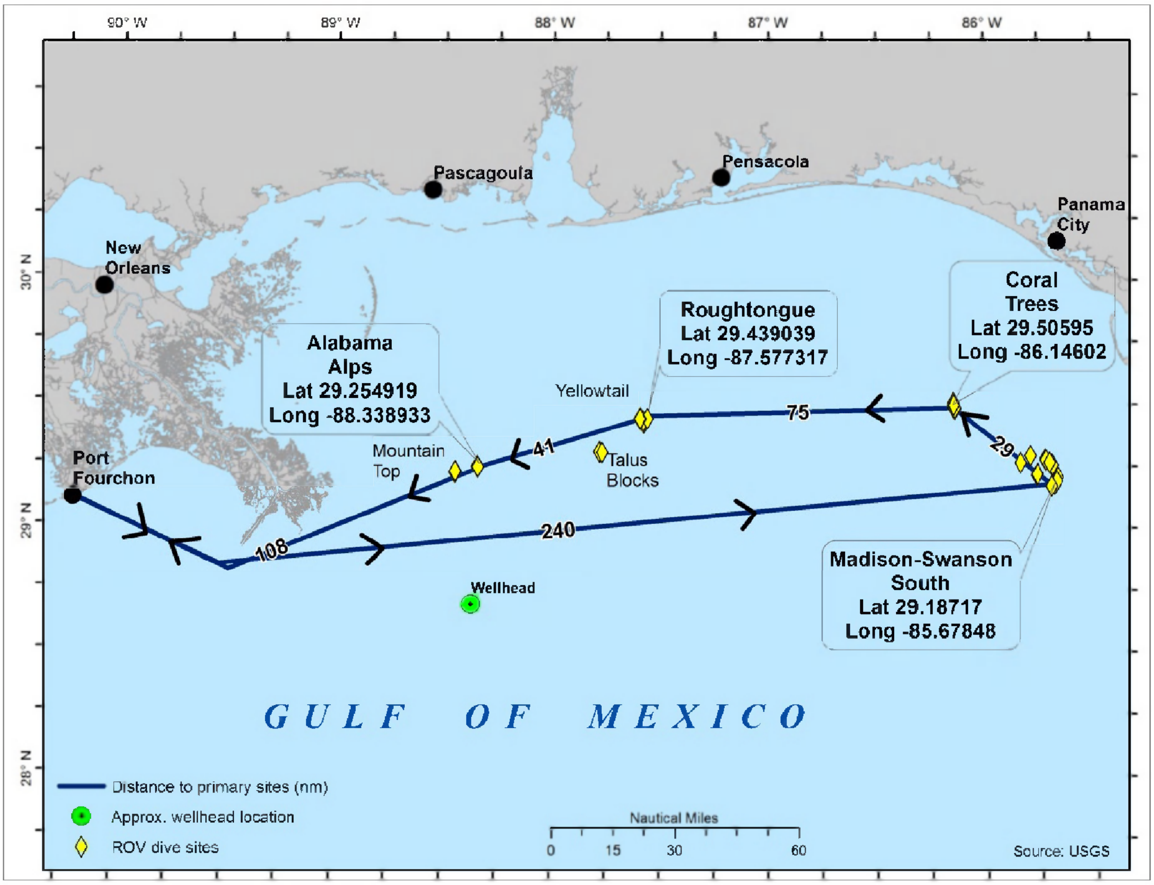


Fig. 5. Schematic cruise track for NRDA 2011 Mesophotic Reef mission, with approximate lat long coordinates provided for convenience (refer to Appendix B for precise coordinates of potential station locations, and to Appendix D for individual primary reef site maps).

Digital and Shipboard Data

All data including imagery (to include navigation, photographic, ARC-GIS layers, and multibeam raw data files, instrument data, field logs and documentation) and all other electronic data will be saved to an on-board computer, and all data shall be migrated to several dedicated external hard drives, providing multiple copies for NRDA and backup. The data will be controlled and managed under project protocols, including Chain-of-Custody tracking of the external hard-drives. Upon return to port, the vessel Operations Manager shall produce identical copies of the raw and processed electronic media generated during the cruise and deliver one of those copies each to NOAA (or its QA contractor) and to BP/Cardno ENTRIX. Additionally, all non-analytical data, including field reports and data sheets, will be made available to BP/Cardno ENTRIX and the Louisiana Oil Spill Coordinator’s Office (LOSCO) within 45 days after completion of the cruise.

Laboratory Data

Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the Trustee Data Management Team (DMT), the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and to BP (or Cardno ENTRIX on behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the Trustees' Data Management Team (DMT). Any preliminary data distributed to the DMT shall also be distributed to LOSCO and to BP (or Cardno ENTRIX on behalf of BP). Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Quality Assurance Project Plan, after which time the validated/QA/QC'd data shall be made available simultaneously to all Trustees and BP (or Cardno ENTRIX on behalf of BP). Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Quality Assurance Project Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated/QA/QC’d data set released by the DMT shall be considered the consensus data set. In order to assure reliability of the consensus data and full review by the parties, no party shall publish consensus data until 7 days after such data has been made available to the parties. Also, the LADP shall not be released by the DMT, LOSCO, BP or Cardno ENTRIX prior to validation/QA/QC absent a showing of critical operational need. Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/unvalidated" and will be made available equally to all trustees and to BP (or Cardno ENTRIX on behalf of BP).

Retention of Sample Materials

All materials associated with the collection or analysis of samples under these protocols or pursuant to any approved work plan, including any remains of samples and including remains of extracts created during or remaining after analytical testing, must be preserved and disposed of in accordance with the preservation and disposal requirements set forth in Pretrial Orders (“PTOs”) # 1, # 30, #35, # 37, #39 and #43 and any other applicable Court Orders governing tangible items that are or may be issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010). Destructive analytical testing of oil, dispersant or sediment samples may only be conducted in accordance with PTO # 37, paragraph 11, and PTO # 39, paragraph 11 and any other applicable Court Orders governing destructive analytical testing that may be issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010). Circumstances and procedures governing preservation and disposal of sample materials by the trustees must be set forth in a written protocol that is approved by the state or federal agency whose employees or contractors are in possession or control of such materials and must comply with the provisions of PTOs # 1, # 30, # 35, 37, #39 and #43.

Chain of Custody

All data collected pursuant to this scope of work must adhere to a strict Chain of Custody protocol to ensure the utmost integrity of all data, methods, control and documentation. All data will remain in the documented physical control of the selected contractors at all times. Complete documentation of this Chain of Custody must follow the standard NRDA Chain of Custody for seafloor imagery, including acceptance and release signature for this physical control chain.[5] Original copies of all documentation will be provided to the signatories, or their designated representative in accordance with this section.

Reporting and Deliverables: Within one month of signature of this plan, the Trustee scientists will deliver a summary cruise report. Within six months of the signature of this plan the Trustee scientists will prepare a final report. Appendix G describes the contents of each of these reports. Raw data will include tender vessel station data, specimen collection data, sediment collection data, and ROV CTD data, as well as any additional raw data obtained during the 2011 mission. Raw data will be accompanied by a copy of all video and still imagery obtained, provided to BP at the end of the cruise on a hard-drive. Raw data will be accompanied by a copy of all video and still imagery obtained.

BP/Cardno ENTRIX Participation in Field Efforts: Reasonable provision shall be made for a sufficient number of BP/Cardno ENTRIX representatives to participate in field efforts, during proposed working hours. For 24-hour operations, 2-3 representatives may be required.

Safety Plans: A HASP binder containing all health and safety protocols will be provided to the vessel. Principal investigators will merge any applicable NRDA health and safety plans with any applicable university or participating organization practices. All NRDA and study-specific safety protocols will be followed.

VII. LOGISTICS:

Mobilization and Cruise Schedule

Proposed cruise dates are September 15-30, 2011. Twenty-four hour operations are anticipated for this study. USGS lead scientific/technical personnel will conduct a pre-cruise OSV and ROV configuration evaluation site visit in the OSV home port, Port Fourchon, LA, on 5 September 2001. USGS scientists and assistants and collaborating scientists from other institutions will assemble gear and deploy to the OSV home port for pre-cruise mobilization to take place 15 September 2011. Some advance equipment and supplies will be shipped to the OSV operator in advance of mobilization. The 14-day at-sea mission will deploy after 00:01, 16 September 2011, making way directly to the MSSR reference site on the West Florida shelf edge (refer to Methods, Appendix B, and the schematic cruise track, Fig. 5). Mobilization and sampling efforts are scheduled for mid-September 2011. One day in-port mobilization time will be required to load scientists and scientific gear, integrate instrumentation with the operational platforms and to establish and test real-time communication between surface vessels and deep-sea equipment. During the ca 24-hr transit from Port Fourchon to the initial MSSR site dive location, final pre-

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[5] Refer to the Work Plan entitled "Technical Specifications and Scope of Work/Services for Aerial Image Acquisition and Image Processing in Support of the MC252 NRDA Process, Fall 2010 Through Spring 2012" (October 6, 2010 version) for more details and for a copy of the COC form.

dive ROV configuration, gear testing, computer set-up, communications linkages, and other at-sea sampling and data/specimen management details will be fine-tuned. Twelve days of at-sea sampling time will be scheduled to allow sufficient time to conduct the mission, meet the mission objectives, and address potential issues with equipment and weather. This includes an estimated two days of transit time between port and the sampling sites and return to port. Aside from any unexpected deviations due to weather or gear exigencies, the mission will follow the schematic cruise track shown in Fig. 5, in order of the primary reef study sites: MSSR, CT, RTR and AAR. The candidate ROV dive locations at each of these primary study reef sites are shown in the series of close-up maps that comprise Appendix D. The mission will demobilize upon return to the OSV home port.

Vessels

The OSV *Holiday Chouest* is a vessel in the NRDA fleet that is available and will be equipped to conduct the proposed sampling. The Holiday Chouest is equipped with a Schilling ROV. A CTD, dissolved oxygen sensor, fluorometer, and turbidity sensor will be mounted on the ROV. In addition to the suite of oceanographic instruments, the ROV will be equipped with live-feed video TV cameras, high quality zoomable digital still cameras mounted for variable pan and tilt, appropriate lighting for imagery, and an HD video system that will provide real-time, *in situ* images of the underwater environment. The ROV will also be equipped with specimen collection devices including a suction sampler and a biobox, appropriately constructed to protect delicate invertebrate specimens from damage due to bottom sediments, currents, surface wave action, air desiccation during surface retrieval, and thermal impacts in warm surface waters during ROV retrieval. The ROV will be configured so as to accommodate 12 sediment push-core samplers, stored in vertical quivers.

At-Sea Transfer of Samples

No at-sea transfer of samples are anticipated for this study.

Sampling Equipment and Containers

The number and type of containers required for the sediment and tissue samples are detailed below. Specialty equipment required for sample collection and preservation are also listed below.

Specialty Equipment:

- Sediment push-cores deployed by the ROV. The coring system consists of core tubes (6.35 cm inside diameter) capable of collecting core samples down to a sediment depth of approximately 30 cm.
- Freezing at -20 degrees C.

Sample Containers (per sample):

- Sediment: 1 sterile 125 mL jar with Teflon lid.
- Tissue (or whole specimen): 1 sterile 1 L jar with Teflon lid or solvent rinsed foil.

VIII. LITERATURE CITED:

Andrews, A. H., E. E. Cordes, M. M. Mahoney, K. Munk, K. H. Coale, G. M. Calliet, J. Heifetz. 2002. Age, growth and radiometric age validation of a deep-sea, habitat-forming gorgonian (Primnoa resedaeformis) from the Gulf of Alaska. Hydrobiologia 471(1):101-110.

Cooksey, C., J. Hyland, and M. Fulton. 2010. Cruise Report: Regional Assessment of Ecosystem Condition and Stressor Impacts along the Northeastern Gulf of Mexico Shelf. NOAA Technical Memorandum NOS NCCOS 121. 73 pp.

Gardner, J. V., J. E. H. Clarke, L. A. Mayer, and P. Dartnell. 2003. Bathymetry and acoustic backscatter of the mid and outer continental shelf, head of DeSoto Canyon, northeastern Gulf of Mexico. U.S. Geological Survey Open-File Report OF03-007, CD-ROM; online at http://geopubs.wr.usgs.gov/ openfile/of03-007. [

Gardner, J. V., P. Dartnell, and K. J. Sulak. 2002. Multibeam mapping of the Pinnacles Region, Gulf of Mexico. U.S. Geological Survey Open-File Report OF02-6, CD-ROM; online at http://geopubs.wr.usgs.gov/ openfile/of02-6.

Gardner, J. V., K. J. Sulak, P. Dartnell, L. Hellequin, B. Calder, and L. A. Mayer. 2000. The bathymetry and acoustic backscatter of the Pinnacles area, northern Gulf of Mexico. U.S. Geological Survey Open-File Report 2000-350, 35 pp.

Goffredo S., Lasker H. R. 2006: Modular growth of a gorgonian coral can generate predictable patterns of colony growth. Journal of Experimental Marine Biology and Ecology, 336: 221-229.

NOAA. 2011. Mississippi Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment, Analytical Quality Assurance Plan, Version 2.2, January 20, 2011.

NOAA Ocean Explorer Website [http://oceanexplorer.noaa.gov] 2002. NOAA Ocean Explorer: Islands in the Stream 2002: Exploring Underwater Oases (Leg 2). Logs for 6-15 August 2002.

Sulak, K. J., R. A. Brooks, K. E. Luke, A. D. Norem, M. Randall, A. J. Quaid, G. E. Yeargin, J. M. Miller, W. M. Harden, J. H. Caruso, and S. W. Ross. 2007. Demersal fishes associated with *Lophelia pertusa* coral and hard-substrate biotopes on the continental slope, northern Gulf of Mexico, pp 65-92, IN: Conservation and Adaptive Management of Seamount and Deep-Sea Coral Ecosystems (R. Y. George and S. Cairns, eds.). Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL, November 2007.

Sulak, K. J., J. Berg, M. Randall, G. D. Dennis III, and R. A. Brooks. 2008a. Dual-Carbon Sources Fuel the Deep Reef Community, a Stable Isotope Investigation. Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008, pp 890-894.

Sulak, K. J., M. T. Randall, K. E. Luke, A. D. Norem, and J. M. Miller. 2008b. Characterization of Northern Gulf of Mexico Deepwater Hard Bottom Communities with Emphasis on *Lophelia* Coral - *Lophelia* Reef Megafaunal Community Structure, Biotopes, Genetics, Microbial Ecology, and Geology. USGS Open-File Report 2008-1143, and OCS Study MMS 2008-015, 15 April 2008, 418 pp. + 8 appendices (incl. interactive DVD). Available in DVD format (2 disks) and online in Adobe® .pdf and .html formats at: http://fl.biology.usgs.gov/coastaleco/OFR_2008_1148_MMS_2008_015/index.html.

Thayer, G. W., J. J. Govoni, and D. W. Connally. 1983. Stable carbon isotope rations of the planktonic food web in the northern Gulf of Mexico. Bulletin of Marine Science 33(2):247-256

Weaver, D. C. G. D. Dennis III, and. K. J. Sulak. 2002. Community structure and trophic ecology of demersal fishes on the Pinnacles Reef tract. U.S. Department of the Interior, U.S. Geological Survey Biological Sciences Report USGS BSR 2001-0008; Minerals Management Service, OCS Study MMS-2002-034. http://fl.biology.usgs.gov/coastaleco/Tech-Rept-Pinnacles-2002/tech-rept-pinnacles-2002.html.

IX. ESTIMATED BUDGET – 2011 MESOPHOTIC REEF INVESTIGATION

The costs summarized below are associated with the collection of the ROV data collected during this project. Trustees' costs are in Budget Chart #1. BP shall reimburse the field survey costs, miscellaneous costs, and travel costs indicated in Budget Chart # 1 below upon receipt of written invoices submitted by the Trustees.

The Parties acknowledge that this budget is an estimate, and that actual costs may prove to be higher. BP's commitment to fund the costs of this work includes any additional reasonable costs within the scope of this approved work plan that may arise. The Trustees will make a good faith effort to notify BP in advance of any such increased costs.

Budget Chart #1:

A: LABOR, SUPPLIES, TRAVEL, AND REPORTING

1. SCIENTIST SALARY COSTS (PRE-MISSION, AT-SEA, IN-LAB)

Person	Role	Amount ($)
Sulak, K.	Scientist, PI, Fish Community	75,000
Demopoulos, A.	Scientist, Co-PI, Benthic Ecology Sediment Collection	10,000
Randall, M.	Scientist, Assisting Fish Biologist	50,000
Adler, J., Res. Asst., Jacobs Contract	Research Assistant, ROV video transect analysis	70,000
Price, M., Res. Asst. Jacobs Contract	Research Assistant, ROV video transect analysis	70,000
Foster, A.	GIS Specialist, transect mapping	15,000
MacDonald, I.	Time-lapse Photography	40,000
TBA Technicians (7) [MacDonald]	General Sampling and Time-lapse Photography	100,000

TBA Technicians (3) [Demopoulos]	Benthos Assisting Technicians [combined]	40,000
Technician (1) [Rittinghouse]	Coral Assisting Technician On behalf of P. Etnoyer, NOAA	50,000

SUBTOTAL, SALARY COSTS **520,000**

2. SUPPLIES & SERVICES

	Specs.	Amount
SD deck		2,000
SD cards	3 hr/each; 14 sea days; 4 per day; 53 total	3,000
Video storage – portable hard-drives (5)		1,000
Shipping of USGS cruise gear to/from RV mobilization port		500
Sample preparation and preservation supplies (vials, jars)		500
Post-cruise disposal fee for used chemical preservatives		500
CDOM meter, Wetlabs, CTD 02 probe calibration, Seabird Electronics		1,000
Cruise preparation materials		1,000
Etnoyer - coral specimen processing supplies		1,500
France, ULL, coral specimen ID & curation (100 samples)		20,000
Florida State University, Ethernet Camera & supplies use costs (Dr. Ian MacDonald)		8,000

SUBTOTAL SUPPLIES & SERVICES **39,000**

3. TRAVEL & ACCOMMODATION

USGS Personnel, Cruise, Conference & Consultation	3,000
MacDonald Lab Personnel	6,000
NOAA Collaborator Travel (Etnoyer technician,	2,000

Harter)

SUBTOTAL TRAVEL & ACCOMMODATION	**11,000**
4. REPORT PREPARATION	**8,000**
TOTAL DIRECT COSTS	**578,000**
INDIRECT COSTS (USGS, 33% OF DIRECT)	**190,740**
TOTAL LABOR, SUPPLIES, TRAVEL, AND REPORTING	**768,740**

APPENDIX A: MESOPHOTIC REEF FISH COMMUNITY ROV SURVEY & ANALYSIS METHODOLOGY (2011, MODIFIED FROM FUNDAMENTAL 2010 METHODS)

2011 ROV TRANSECT & IMAGERY METHODS. AT-SEA:

A. Primary study reefs will be the two 2010 test reefs (Alabama Alps Reef [AAR] and Roughtongue Reef [RTR] in the LA/MS/AL Pinnacles shelf-edge reef tract), and the two reference sites (Madison-Swanson South Ridge & Coral Trees [CT] complex on the West Florida shelf-edge). Refer to Cruise Track Map (above) and individual study site station location planning maps (Appendix D). Contingency test and reference sites (should vessel traffic, commercial fishing operations, naval operations, etc. preclude diving on the primary study reefs) will be Yellowtail Reef, Mountain Top Reef, Deep Reef A (Talus Block Reef) and Madison-Swanson Inside/Outside Reefs. Coordinates of all potential ROV dive sites are given in Appendix Table B below. The basic objective is to conduct approximately 3 days of ROV dives at each of the two test reef sites, and also at the comparative reference site.

B. ROV dive sites on AAR and RTR will repeat the starting positions of 2010 mission ROV dive sites. Additional ROV dive site starting coordinates will repeat the starting positions of prior USGS 1997-2003 ROV dives (Appendix Table B below). Existing USGS high-resolution multibeam bottom topography imagery will be used to inform selection of additional ROV dive sites. This will insure that all dives are conducted on comparable reef-top biotope, as classified in previous USGS ROV missions. Dives will not target other reef and/or reef-associated biotopes (reef-base sedimentary apron, reef slope, and reef crest).

C. ROV dives will be accomplished in both daytime and night-time. This will address a deficiency from 2010 when only daytime dives were conducted, allowing the potential of an undetected shift in planktivore fish activity patterns.

D. Dive positions and ROV transects will be monitored and mapped real-time using ARC-GIS in combination with feeds from the bridge and from ROV coms.

E. The maintenance and support vessel (MSV) will launch the ROV at the established dive position. Upon reaching bottom, a video start position will be determined. If repeating a 2010 ROV dive, the transect compass direction will duplicate the 2010 compass direction. If the dive is launched at a new (non-2010) site, a set of computer-generated random compass headings (001-360 degrees) will be consulted to select the first heading that will enable a 5-min transect over reef-top habitat. A consistent heading will be followed as far as terrain permits. Each transect that exceeds a 4.0 min threshold will be accepted as a full transect. Maximum transect duration will be 5.0 min. If a transect must be halted prior to 4.0 min due to terrain, or other logistic exigency, a second segment will be undertaken and combined with the first segment to achieve a 4.0 – 5.0 min total transect time.

F. During video transecting, the videocamera pan, tilt and zoom will be held constant at pre-set positions, pan held straight forward, zero degrees, tilt set to 45 degrees down, and zoom set on full wide angle. ROV speed over bottom will be maintained at the minimum achievable, targeting a range of 0.1-0.2 knots. ROV altitude will be maintained at a minimum, just skimming over the reeftop terrain. The ship will be re-positioned, as necessary, during each dive, to enable transects to span a horizontal distance exceeding the length of the tether/communication umbilical between the down-weight (clump-weight) and the ROV.

G. At the completion of each nominal 5-min video transect, a 25-min round of close-up video imagery, digital imagery, and invertebrate specimen collection will be undertaken to critically identify fish and invertebrate species, document the diversity of the fauna, document the condition of dominant sessile invertebrates, and obtain voucher specimens to enable laboratory validation of taxonomic identifications. Both the ROV fixed-mount and MacDonald Ethernet digital cameras will be employed. Then the cycle of alternating 5-min transects and 25-min close-up imagery will repeat until completion of ROV operations for each 6-hr bottom time period. Thus, each 6-hr span of ROV operations should result in up to 12 nominal 5-min standardized quantitative video transects. Due to various logistic exigencies, including technical problems and weather, a target of 8-10 transects per 6-hr period is more realistic. The ROV work day will be 24 hours. Two 12-hr periods of ROV dive operations (including downtime, deployment and retrieval) will be conducted during each 24-hr period. Thus ROV operations will span both the period of daytime (the predicted period of fish planktivore maximum activity), and the period of nighttime (predicted time of minimum fish planktivore activity – but maximum coral feeding activity).

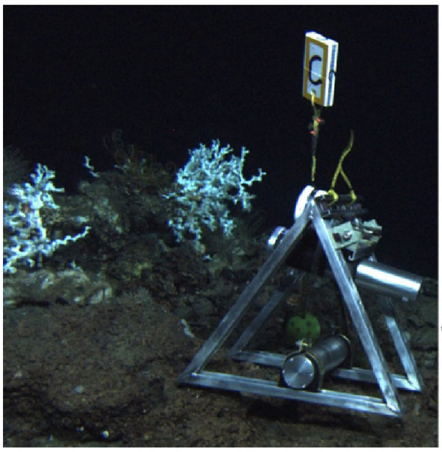
H. The ROV down-weight module will be equipped with a mini-CTD to monitor and record standard water parameters, including temperature, depth, conductivity, and optical transmissivity. The CTD data will be continuously recorded via real-time feed.

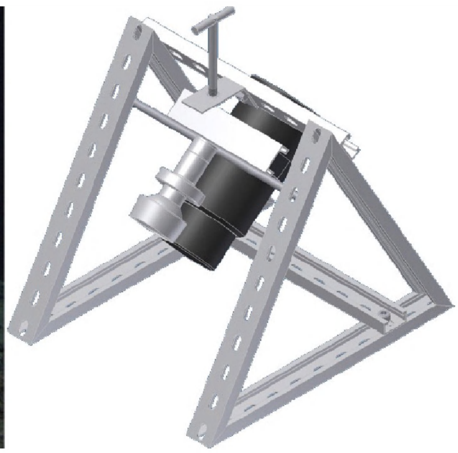
I. In the ship's laboratory, the real-time HDTV video feed from the ROV will be recorded directly to a HDTV recorder, and simultaneously recorded to a SD recorder. The HDTV tape recording will not include the date, time, depth, altitude, compass direction overlay to prevent occlusion of target organisms. However, to coordinate subsequent time-referenced video analysis in the laboratory, that data overlay will be recorded to SD card. Each dive will be recorded on both HDTV tapes and SD cards (32gb) and will be appropriately labeled with the established station/dive identification number. The SD card record, with overlaid data inset, will also be backed up to two 2-tb portable hard-drives, to serve as a second separate back-up of the video imagery.

J. The real-time HDTV video feed from the ROV will be continuously viewed on a 48" class plasma monitor – attended by 1-2 scientists or assistants, who will record (hardcopy in pencil on standard forms) time-referenced fish species identifications and reef habitat type during each dive, during both timed transect segments and all other segments. Hardcopy documents will be scanned to pdf after each dive to provide a computer-stored archive of hardcopy records. A coral specialist will simultaneously view the video feed to assess coral health and condition.

2011 COMPLEMENTARY DEPLOYABLE BOTTOM DIGITAL STILL CAMERA IMAGERY METHODS. AT-SEA:

Dr. Ian MacDonald, Florida State University, will provide two automated, deployable, digital still and video bottom cameras (refer to attached images below showing two camera deployment configurations). The upright rotary time-lapse camera has 8” x 8” x 36” dimensions and weighs ~30 lbs. in air (5-10 lbs. in water because of the float). The upright rotary system configuration will cover a wide habitat area for faunal documentation. It rotates 360° in stages on an interval schedule (10 images per hr.). The frame-mounted oblique-looking video camera will home in on a given area of potential interest to document diurnal/nocturnal behavior patterns. The video system has a 20” x 14” x 17" footprint and weighs ~25 lbs. in air. The camera pair will be deployed for a span of 2 days at each primary study site. Cameras will be deployed on day-1 at a given reef site, and retrieved on a subsequent ROV dive approximately 48 hours later. The still camera will take a large sequence of digital still images at intervals. This will provide an independent, low-disturbance, evaluation of reef fish planktivore activity and abundance, in the absence of potential ROV disturbance of the fauna. The bottom camera(s) will be deployed on day-1 at a given primary reef study site, following the initial 3-hr ROV recon dive. During that initial recon dive, a suitable area of well-developed reef biotope, suitably elevated to enable 360° visibility, will be chosen and marked as the site for deployment of the bottom camera on the subsequent ROV dive.





2011 VIDEO & STILL DIGITAL ANALYSIS METHODS. IN-LABORATORY:

A. In the USGS Gainesville laboratory, the HDTV video imagery from each ROV dive will viewed on a 48” class plasma flat screen monitor in a darkened room to minimize screen glare. An initial simultaneous screening of the HDTV tapes (to provide highest quality in species identifications) and SD card recordings (to provide the time line) will be undertaken to view each transect, record SD card recording transect start and end times to coordinate with HDTV tape run times, combine selected transect segments of short duration into combined transects of minimum 4.0-min total duration, and to identify all fish taxa imaged during both transect and non-transect video segments to build a working taxonomic list for subsequent quantification. Start and stop times defining transects will be shared with BP/Cardno ENTRIX to facilitate discussions regarding establishing an agreed-upon set of transects for interpretive analysis.

B. A second screening of all transects will be undertaken to identify each fish image both while in motion and while stopped (freeze frame), utilizing size, color, markings, behavior, swimming motion, and habitat affinity. This second screening will establish the fish species list for each transect, and the overall list of fish taxa recorded from all transects. For fish identification validation, 8.5” x 11.0” color prints were prepared in 2010 from the USGS archive of layout images of each fish species. These prints will be posted on the walls of the viewing room for ready reference. The project PI (Sulak) will provide definitive validation of fish species identifications.

C. A final video screening will be undertaken to enumerate fish specimens imaged during each quantitative transect. This work typically requires two scientists working in conjunction to simultaneously view and record while attending as well to the tape time. Each specimen will be recorded according to either the real-time (at-sea, video data overlay) or tape run time (in-lab VCR run time) reference (hh:mm:ss). Only individuals crossing (moving out of view) the bottom, left or right margins of the video screen will be enumerated as the video tape is advanced. Fish specimens entering the field of view from the left, right, or bottom (i.e., potentially re-entering the counting frame) will not be enumerated to avoid potential duplicate counts of the same fish. Specimens will be recorded by time reference onto hardcopy sheets. These tally sheets will be scanned as individual pdf files which will be archived and backed-up.

D. For each transect or transect segment completed, an entry will be made in a bound hardcopy log to document completion of transect quantitative analysis. The scientist doing the transect imagery analysis will sign the log to certify completion of video analysis of that transect. Each transect will be identified by the videotape number, reef site, and start and end time. This pdf scans of the hardcopy logs will be archived and backed-up.

E. An Excel® spreadsheet file will also be prepared to cross-reference the RV station numbers, ROV dive numbers, at-sea transect designations, and final in-laboratory transect identification numbers, and to document progress in completion of analysis of the full set of video transects for the 2011 mission.

2011 VIDEO TRANSECT ANALYSIS METHODS – IN LABORATORY:

A. Count data for all transects comprising the set of samples for each target test and reference reef site will be arrayed (against the full list of NEGOM reef fish taxa from all previous USGS ROV studies at all previously studied reef sites across the NEGOM, LA to FL, Weaver et al. 2002) in an Excel spreadsheet. The full USGS NEGOM deep shelf-edge reef fauna fish species list contains some species that were not recorded in 2010 and may not be observed in 2011 at the selected test and reference reef sites. Thus, some species in the individual transect lists or summaries may receive no scores.

B. Fish data will be summarized in Excel by study reef (MSSR and CT reference reef control sites, AAR Reef test site, RTR test site) and by selected functional (trophic) group (All Taxa, Anthiine Planktivores, Large Perciform Predators, Epifaunal Browsers, and Mid-Sized Opportunistic Predators). Soft corals will be summarized by genus (e.g., Hypnogorgia, Swiftia, Thesea, Bebryce) and by presence and absence of observable abnormalities.

C. Paired contrasts will be undertaken between the results for the reference control reef versus each of the two target test reefs using SigmaStat® software. The t-test will be used if a normality test is passed, allowing for parametric contrast. If the normality test fails, the Mann-Whitney Rank Sum test will be used for non-parametric contrast. The criterion for significant differences will be $p<0.05$.

D. Species accumulation curves for paired contrasts, test reef versus control reef, will be prepared using EstimateS® software. This software makes large numbers of random draws upon the data to construct a species accumulation curve (number of species as a function of number of specimens) that is amenable to estimation of variance about the estimated number of species data points. Statistical differences can be determined when the 95% CIs of two such curves fail to overlap, beyond the inflexion points of comparative curves.

APPENDIX B: MESOPHOTIC REEF FISH MISSION: LIST OF ALL POTENTIAL ROV TARGET STATION LOCATIONS KEY: 'Target' sites are potential new ROV station locations; all others are locations repeating either 2010 mission ROV dive stations or previous USGS pre-2010 ROV dive station locations.

REEF SITE NAME	TYPE	LAT	LONG	DEPTH (m)	CRUISE	STATION NUMBER (GLOB. EXPL. DIVE #)	2010 VIDEOTAPE # (AT SEA)	2010 LAB TRANSECT # (ANALYZED)
ALABAMA ALPS REEF A	TARGET	29.25350	88.33850					
ALABAMA ALPS REEF B	TARGET	29.25183	88.33733					
ALABAMA ALPS REEF C	TARGET	29.25600	88.33883					
ALABAMA ALPS REEF D	TARGET	29.25183	88.33950					
ALABAMA ALPS REEF	2003	29.24985	88.33668	83	USGS-2003-01	2003-01-0103		
ALABAMA ALPS REEF	1999	29.25317	88.33933	69	USGS-1999-03	1999-03-2113 (211A)		
ALABAMA ALPS REEF	1999	29.25317	88.33933	73	USGS-1999-03	1999-03-2114 (211B)		
ALABAMA ALPS REEF	1999	29.25383	88.33967	70	USGS-1999-03	1999-03-0212		
ALABAMA ALPS REEF	2000	29.25167	88.33833	70	USGS-2000-01	2000-01-0033		
ALABAMA ALPS REEF	2000	29.25113	88.33827	69	USGS-2000-01	2000-01-0036		
ALABAMA ALPS REEF	2000	29.25098	88.33895	67	USGS-2000-01	2000-01-0038		
ALABAMA ALPS REEF	2010	29.25458	88.33938	73	USGS-2010-01	2020-01-1081 (GE 013)	2010-08-06-A-HD2-01 TRANSECT 1 AA	2010-08-06-AA-T1
ALABAMA ALPS REEF	2010	29.25492	88.33893	72	USGS-2010-01	2020-01-1083 (GE 013)	2010-08-06-A-HD2-02 TRANSECT 2 AA	2010-08-06-AA-T2
ALABAMA ALPS REEF	2010	29.25455	88.33952	73	USGS-2010-01	2020-01-1085 (GE 013)	2010-08-06-A-HD2-03 TRANSECT 3 AA	2010-08-06-AA-T3A
ALABAMA ALPS REEF		29.25484	88.33949	72				2010-08-06-AA-T3B
ALABAMA ALPS REEF	2010	29.25448	88.33973	70	USGS-2010-01	2020-01-1086 (GE 013)	2010-08-06-A-HD2-04 TRANSECT 4 AA	2010-08-06-AA-T4
ALABAMA ALPS REEF	2010	29.25137	88.33789	74	USGS-2010-01	2020-01-1092 (GE 015)	2010-08-07-A-SD2-01 TRANSECT 1 AA	2010-08-07-AA-T1
ROUGHTONGUE REEF A	TARGET	29.43800	87.57550					

ROUGHTONGUE REEF B	TARGET	29.43500	87.57583			
ROUGHTONGUE REEF C	TARGET	29.43967	87.57500			
ROUGHTONGUE REEF D	TARGET	29.44150	87.57610			
ROUGHTONGUE REEF E	TARGET	29.43933	87.57620			
ROUGHTONGUE REEF F	TARGET	29.43850	87.57600			
ROUGHTONGUE REEF G	TARGET	29.43867	87.57417			
ROUGHTONGUE REEF	1997	29.43672	87.57447	68	USGS-1997-01	1997-01-0009
ROUGHTONGUE REEF	1997	29.43907	87.57642	66	USGS-1997-01	1997-01-0011
ROUGHTONGUE REEF	1997	29.44000	87.57717	69	USGS-1997-01	1997-01-0012
ROUGHTONGUE REEF	1997	29.43837	87.57627	71	USGS-1997-01	1997-01-0065
ROUGHTONGUE REEF	1997	29.43822	87.57567	65	USGS-1997-01	1997-01-0069
ROUGHTONGUE REEF	1999	29.43650	87.57713	76	USGS-1999-02	1999-02-0006
ROUGHTONGUE REEF	1999	29.43933	87.57617	62	USGS-1999-03	1999-03-0214
ROUGHTONGUE REEF	2000	29.43883	87.57600	62	USGS-2000-01	2000-01-0012
ROUGHTONGUE REEF	2000	29.43843	87.57540	62	USGS-2000-01	2000-01-0016
ROUGHTONGUE REEF	2001	29.43955	87.57842	75	USGS-2001-01	2001-01-1061
ROUGHTONGUE REEF	2001	29.43480	87.57102	81	USGS-2001-01	2001-01-1005
ROUGHTONGUE REEF	2001	29.43483	87.57102	81	USGS-2001-01	2001-01-1006
ROUGHTONGUE REEF	2001	29.43862	87.57495	64	USGS-2001-01	2001-01-1040
ROUGHTONGUE REEF	2001	29.44043	87.57502	73	USGS-2001-01	2001-01-1079
ROUGHTONGUE REEF	2001	29.43725	87.57460	74	USGS-2001-01	2001-01-1082
ROUGHTONGUE REEF	2001	29.43890	87.57540	66	USGS-2001-01	2001-01-1101
ROUGHTONGUE REEF	2001	29.44102	87.57623	77	USGS-2001-01	2001-01-1103
ROUGHTONGUE REEF	2001	29.43750	87.57693	77	USGS-2001-01	2001-01-1125
ROUGHTONGUE REEF	2001	29.43940	87.57498	64	USGS-2001-01	2001-01-1159

ROUGHTONGUE REEF	2001	29.43733	87.57482	64	USGS-2001-01	2001-01-1193		
ROUGHTONGUE REEF	2001	29.43755	87.57407	73	USGS-2001-01	2001-01-1044		
ROUGHTONGUE REEF	2003	29.43915	87.57683	66	USGS-2003-01	2003-01-0059		
ROUGHTONGUE REEF	2003	29.43915	87.57738	64	USGS-2003-01	2003-01-0060		
ROUGHTONGUE REEF	2010	29.43899	87.57732	66	USGS-2010-01	2010-01-1023 (GE 009)	2010-08-03-A-HD2-01 TRANSECT 1 RTR	2010-08-03-RTR-T1
ROUGHTONGUE REEF	2010	29.43900	87.57732	66	USGS-2010-01	2010-01-1024 (GE 009)	2010-08-03-A-HD2-01 TRANSECT 2 RTR	2010-08-03-RTR-T2
ROUGHTONGUE REEF	2010	29.43908	87.57702	69	USGS-2010-01	2010-01-1025 (GE 009)	2010-08-03-A-HD2-02 TRANSECT 3 RTR	2010-08-03-RTR-T3
ROUGHTONGUE REEF	2010	29.43910	87.57777	66	USGS-2010-01	2010-01-1027 (GE 009)	2010-08-03-A-HD2-02 TRANSECT 4 RTR	2010-08-03-RTR-T4
ROUGHTONGUE REEF	2010	29.43895	87.57757	66	USGS-2010-01	2010-01-1030 (GE 009)	2010-08-03-A-HD2-02&03 TRANSECT 5 RTR	2010-08-03-RTR-T5
ROUGHTONGUE REEF	2010	29.43891	87.57778	67	USGS-2010-01	2010-01-1031 (GE 009)	2010-08-03-A-HD2-03 TRANSECT 6 RTR	2010-08-03-RTR-T6
ROUGHTONGUE REEF	2010	29.43893	87.57738	66	USGS-2010-01	2010-01-1032 (GE 009)	2010-08-03-A-HD2-04 TRANSECT 7 RTR	2010-08-03-RTR-T7
ROUGHTONGUE REEF	2010	29.43904	87.57732	67	USGS-2010-01	2010-01-1033 (GE 009)	2010-08-03-A-HD2-04 TRANSECT 8 RTR	2010-08-03-RTR-T8
ROUGHTONGUE REEF	2010	29.43914	87.57724	67	USGS-2010-01	2010-01-1034 (GE 009)	2010-08-03-A-HD2-04 TRANSECT 9 RTR	2010-08-03-RTR-T9
ROUGHTONGUE REEF	2010	29.43926	87.57724	67	USGS-2010-01	2010-01-1035 (GE 009)	2010-08-03-A-HD2-04 TRANSECT 10 RTR	2010-08-03-RTR-T10
ROUGHTONGUE REEF	2010	29.43852	87.57635	65	USGS-2010-01	2010-01-1049 (GE 010)	2010-08-04-A-HD2-01 TRANSECT 1 RTR	2010-08-04-RTR-T1
ROUGHTONGUE REEF	2010	29.43847	87.57564	64	USGS-2010-01	2010-01-1051 (GE 010)	2010-08-04-A-HD2-01&02 TRANSECT 2 RTR	2010-08-04-RTR-T2
ROUGHTONGUE REEF	2010	29.43868	87.57559	64	USGS-2010-01	2010-01-1053 (GE 010)	2010-08-04-A-HD2-02 TRANSECT 3 RTR	2010-08-04-RTR-T3
ROUGHTONGUE REEF	2010	29.43864	87.57526	65	USGS-2010-01	2010-01-1054 (GE 010)	2010-08-04-A-HD2-02 TRANSECT 4 RTR	2010-08-04-RTR-T4

ROUGHTONGUE REEF	2010	29.43886	87.57556	66	USGS-2010-01	2010-01-1055 (GE 010)	2010-08-04-A-HD2-02 TRANSECT 5 RTR	2010-08-04-RTR-T5
ROUGHTONGUE REEF	2010	29.43899	87.57524	65	USGS-2010-01	2010-01-1056 (GE 010)	2010-08-04-A-HD2-03 TRANSECT 6 RTR	2010-08-04-RTR-T6
ROUGHTONGUE REEF	2010	29.43878	87.57539	66	USGS-2010-01	2010-01-1058 (GE 010)	2010-08-04-A-HD2-03 TRANSECT 7 RTR	2010-08-04-RTR-T7
ROUGHTONGUE REEF	2010	29.43891	87.57503	65	USGS-2010-01	2010-01-1059 (GE 010)	2010-08-04-A-HD2-03 TRANSECT 8 RTR	2010-08-04-RTR-T8A
ROUGHTONGUE REEF		29.43885	87.57514	66				2010-08-04-RTR-T8B
ROUGHTONGUE REEF	2010	29.43877	87.57530	65	USGS-2010-01	2010-01-1060 (GE 010)	2010-08-04-A-HD2-03 TRANSECT 9 RTR	2010-08-04-RTR-T9A
ROUGHTONGUE REEF		29.43879	87.57530	66				2010-08-04-RTR-T9B
ROUGHTONGUE REEF	2010	29.43894	87.57541	65	USGS-2010-01	2010-01-1061 (GE 010)	2010-08-04-A-HD2-04 TRANSECT 10 RTR	2010-08-04-RTR-T10
ROUGHTONGUE REEF	2010	29.43908	87.57562	65	USGS-2010-01	2010-01-1062 (GE 010)	2010-08-04-A-HD2-04 TRANSECT 11 RTR	2010-08-04-RTR-T11
YELLOWTAIL REEF	1997	29.44853	87.59028	70	USGS-1997-01	1997-01-0000		
YELLOWTAIL REEF	1997	29.45013	87.59238	63	USGS-1997-01	1997-01-0004		
YELLOWTAIL REEF	1997	29.45037	87.55727	62	USGS-1997-01	1997-01-0083		
YELLOWTAIL REEF	1997	29.44992	87.58840	67	USGS-1997-01	1997-01-0086		
YELLOWTAIL REEF	1997	29.44970	87.59063	63	USGS-1997-01	1997-01-0087		
YELLOWTAIL REEF	1997	29.44988	87.59093	67	USGS-1997-01	1997-01-0090		
YELLOWTAIL REEF	1999	29.45060	87.59115	69	USGS-1997-01	1997-01-000A		
YELLOWTAIL REEF	1999	29.45033	87.59200	58	USGS-1999-03	1999-03-2141 (1997-01-0214A)		
YELLOWTAIL REEF	1999	29.45033	87.59200	58	USGS-1999-03	1999-03-2143 (1997-01-0214B)		
YELLOWTAIL REEF	2000	29.44900	87.59150	59	USGS-2000-01	2000-01-0024		
YELLOWTAIL REEF	2000	29.44998	87.59220	59	USGS-2000-01	2000-01-0028		
YELLOWTAIL REEF	2000	29.45023	87.59267	58	USGS-2000-01	2000-01-0029		
YELLOWTAIL	2001	29.45215	87.59165	65	USGS-2001-01	2001-01-1306		

REEF						
YELLOWTAIL REEF	2001	29.45192	87.59142	68	USGS-2001-01	2001-01-1327
YELLOWTAIL REEF	2001	29.45182	87.59095	69	USGS-2001-01	2001-01-1328
YELLOWTAIL REEF	2001	29.45188	87.59092	69	USGS-2001-01	2001-01-1329
YELLOWTAIL REEF	2003	29.44975	87.59070	60	USGS-2003-01	2003-01-0043
YELLOWTAIL REEF	2003	29.45007	87.59048	60	USGS-2003-01	2003-01-0045
YELLOWTAIL REEF	2003	29.45007	87.59047	60	USGS-2003-01	2003-01-0047
YELLOWTAIL REEF	2003	29.45057	87.59243	59	USGS-2003-01	2003-01-0065
YELLOWTAIL REEF	2001	29.45183	87.59028	65	USGS-2001-01	2001-01-1303
YELLOWTAIL REEF	2001	29.45177	87.59127	66	USGS-2001-01	2001-01-1305
YELLOWTAIL REEF	2001	29.45080	87.59112	64	USGS-2001-01	2001-01-1324
YELLOWTAIL REEF	2001	29.45000	87.59187	65	USGS-2001-01	2001-01-1325
DEEP REFERENCE SITE A	TARGET	29.31422	87.76533			
DEEP REFERENCE SITE B	TARGET	29.31557	87.77573			
DEEP REFERENCE SITE C	TARGET	29.31702	87.77270			
TALUS BLOCK		29.31580	87.77878	134	USGS-2003-01	2003-01-084
TALUS BLOCK		29.31433	87.76512	171	USGS-2003-01	2003-01-086
MOUNTAIN TOP	TARGET	29.23333	88.43750	70		
MOUNTAIN TOP	2003	29.22905	88.44075	58	USGS-2003-01	2003-01-0005
MOUNTAIN TOP	2003	29.22898	88.43950	56	USGS-2003-01	2003-01-0014
MOUNTAIN TOP	2003	29.22833	88.43957	64	USGS-2003-01	2003-01-0017
MOUNTAIN TOP	2003	29.23332	88.43835	62	USGS-2003-01	2003-01-0025
MADISON-SWANSON SOUTH RIDGE	2001	29.17353	85.69700	75	USGS-2001-02	2001-02-1477
MADISON-SWANSON SOUTH RIDGE	2002	29.17360	85.69752	74	USGS-2002-01	2002-01-3098
MADISON-SWANSON	2002	29.17370	85.69778	74	USGS-2002-01	2002-01-3091

SOUTH RIDGE						
MADISON-SWANSON SOUTH RIDGE	**2001**	**29.17373**	**85.69648**	**77**	**USGS-2001-02**	**2001-02-1476**
MADISON-SWANSON SOUTH RIDGE	**2002**	**29.17438**	**85.69688**	**74**	**USGS-2002-01**	**2002-01-3090**
MADISON-SWANSON SOUTH RIDGE	**2001**	**29.18167**	**85.68488**	**75**	**USGS-2001-02**	**2001-02-1478**
MADISON-SWANSON SOUTH RIDGE	**2002**	**29.18717**	**85.67848**	**71**	**USGS-2002-01**	**2002-01-3074**
MADISON-SWANSON SOUTH RIDGE	**2002**	**29.18830**	**85.67823**	**72**	**USGS-2002-01**	**2002-01-3070**
MADISON-SWANSON SOUTH RIDGE	**2001**	**29.18873**	**85.67738**	**70**	**USGS-2001-02**	**2001-02-1425**
MADISON-SWANSON SOUTH RIDGE	**2002**	**29.19125**	**85.68415**	**81**	**USGS-2002-01**	**2002-01-3116**
MADISON-SWANSON SOUTH RIDGE	**2001**	**29.19377**	**85.67402**	**71**	**USGS-2001-02**	**2001-02-1534**
MADISON-SWANSON SOUTH RIDGE	**2001**	**29.20917**	**85.66927**	**69**	**USGS-2001-02**	**2001-02-1533**
MADISON-SWANSON INSIDE	**2001**	**29.21847**	**85.76437**	**83**	**USGS-2001-02**	**2001-02-1503**
MADISON-SWANSON INSIDE	**2001**	**29.22238**	**85.76315**	**92**	**USGS-2001-02**	**2001-02-1532**
MADISON-SWANSON INSIDE	**2001**	**29.22488**	**85.76237**	**98**	**USGS-2001-02**	**2001-02-1504**
MADISON-SWANSON NORTH RIDGE	**2001**	**29.25868**	**85.69268**	**60**	**USGS-2001-02**	**2001-02-1450**
MADISON-SWANSON NORTH RIDGE	**2003**	**29.26218**	**85.69750**	**67**	**USGS-2003-02**	**2003-02-3302**
MADISON-SWANSON NORTH RIDGE	**2001**	**29.26922**	**85.70542**	**75**	**USGS-2001-02**	**2001-02-1451**
MADISON-SWANSON NORTH RIDGE	**2003**	**29.28008**	**85.72273**	**67**	**USGS-2003-02**	**2003-02-3300**

MADISON-SWANSON OUTSIDE	2001	29.29737	85.79460	62	USGS-2001-02	2001-02-1502		
MADISON-SWANSON OUTSIDE	2001	29.26915	85.83962	72	USGS-2001-02	2001-02-1452		
CORAL TREES	2010	29.50504	86.14538	85	USGS-2010-01	2010-01-1010A-B	2010-08-02-CT	USGS-2010-08-02-CT-1010-29AB
CORAL TREES	2010	29.50504	86.14538	85	USGS-2010-01	2010-01-1010C-G	2010-08-02-CT	USGS-2010-08-02-CT-1010-29CG
CORAL TREES	2010	29.50500	86.14600	84	USGS-2010-01	2010-01-1012A-B	2010-08-02-CT	USGS-2010-08-02-CT-1012-30AB
CORAL TREES	2010	29.50345	86.14618	80	USGS-2010-01	2010-01-1013A-E	2010-08-02-CT	USGS-2010-08-02-CT-1013-31AE
CORAL TREES	2001	29.49170	86.13938	81	USGS-2001-02	2001-02-1630		
CORAL TREES	2001	29.49213	86.13945	82	USGS-2001-02	2001-02-1634		
CORAL TREES	2001	29.51398	86.14698	75	USGS-2001-02	2001-02-1658		

APPENDIX C: MESOPHOTIC REEF MISSION, SUPPLEMENTARY METHODOLOGICAL DETAIL FOR ROV OPERATIONS AT SEA.

REFERENCE TARGET STUDY SITE COORDINATES (ship's position on-site for initiation of ROV operations at each Study Site (subject to change), see below for coordinates of all potential ROV video transecting dive stations):

1) **MSSR:** 29.187170 -85.678480
2) **CT** 29.505000 -85.146000
3) **RTR:** 29.439039 -87.577317
4) **AAR:** 29.254919 -88.338933

SPECIFIC ROV VIDEO TRANSECT DIVE STATIONS COORDINATES AT EACH STUDY SITE: Latitude and longitude coordinates for all potential ROV dive stations are listed by study site in Appendix B above. The intent is to conduct 2011 ROV dives so as to repeat 2010 and/or earlier 1997-2003 ROV stations to enable direct comparisons of station-specific fish community structure.

SCIENCE TASK ITINERARY AT EACH PRIMARY STUDY SITE:

1) Sail from Port Fourchon to first target reef site - MSSR reference reef site.
2) En route, configure ROV videocamera, still digital camera, and lasers will be configured as follows to support video transecting for community quantification and fish and sessile invertebrate imagery for identification, as follows:
   a) Primary high quality videocamera will be configured for straight-ahead (pan), 45 degree down oblique tilt), minimal zoom (full wide-angle) transecting. Lighting will be configured to evenly illuminate a minimum 2-3 m wide path ahead of the ROV as it moves at minimum altitude (skimming over the bottom) and very slow speed over ground (0.2 knot). Lasers will be used to ground-truth lateral field of view at beginning of a transect, but not used during transects otherwise. Each bottom ROV transects will follow a linear track, as far as possible, over an interval of 5.0 min. The compass direction of each transect will be determined by a randomly generated course heading – unless the random heading tends off-reef. In that case, the next acceptable random heading will be used. A large series of such standardized, quantitative transects will be accomplished during each period of ROV diving, both day and night. Crane operator works a 12-hr dayshift only, so ROV deployment and retrieval must occur during that shift
   b) A video data overlay showing date, time, and depth (and possibly compass heading) will be configured to facilitate subsequent analysis of the video recordings.
   c) The real-time video output from the ROV videocamera will be recorded and monitored on a large plasma flat screen in the science control van. The HD-SDI video feed will be split. The feed to the HDTV recorder will be 'clean' without the date-time-depth-heading data overlay. The feed to the hard-drive and SD card recorders will have the data overlay recorded with the video stream to enable time-referenced video analysis in the laboratory.
   d) Navigation will be controlled and monitored by Hypak software with ship's and ROV positions being continuously mapped via ArcGIS (USGS GIS specialists).
   e) The primary videocamera will however be mounted such that between sequential transects it can be utilized to pan, tilt and zoom as desired to home in on and obtain high quality close-up imagery of individual target organisms (fishes and invertebrates) for ID

validation. After each 5-min video transect, there will be a 25-min period of close-up specimen and habitat images, during which the ROV and videocamera will be moved as needed to accomplish close-up imagery. Specimens will not be collected during ROV transect and taxon close-up digital still imagery. Except, imagery will be halted if any potentially damaged corals are observed during ROV imaging. Any such specimens will be collected. The number of such collections would be strictly limited to occasional high priority specimens encountered, in order to avoid interference with the primary video transecting and digital still imagery tasks during dives dedicated to imagery.

f) During the 25-min period after each 5-min transect, the high-quality digital camera(s) will also be used to obtain close-up digital imagery of individual target specimens for faunal documentation and ID validation. The dual lasers will be engaged periodically as a scale to determine specimen size. An Ethernet digital still camera, stored on the forward work tray, will also be available for close-up imagery. This will be handled via manipulator.

g) A pair of autonomous bottom cameras will be deployed to the bottom via ROV for a period of 48 hrs., then retrieved. The cameras will operate around the diurnal/nocturnal cycle, capturing images.at pre-set intervals. Image files will be stored and downloaded upon retrieval.

h) An estimated 3-3.5 days of ROV operations are anticipated for each study site. Two days of that time will be devoted to video and still digital imagery with the ROV configured for same. The autonomous bottom cameras will be deployed over the same 2-day time period. The remaining ROV time (1.0-1.5 days) will be devoted invertebrate specimen collection and sediment collection via core tubes. Thus, on day-3 at each study site, the ROV will be configured with the forward work platform (tray) provided with suction sampler, biobox, and/or sediment core tubes. Specimen collection station locations will be chosen from those preceding ROV video dives, using stations offering the highest diversity and abundance to facilitate collection operations. The 7-function manipulator will be engaged to accomplish such physical collections, when the suction sampler is not appropriate to the task. The work day may need to be divided into several-hr. segments with the forward work platform configured specifically for suction sampling and biobox collections, or for sediment coring. This will need to be determined by the ROV tech team during actual sea operations. ROV station locations for sediment collection will be determined in the field with reference to preceding ROV reeftop imaging stations. Sediment collection stations will be accomplished on the periphery of reef habitat, i.e., on the surrounding soft sediment apron. The plan will be to move around the reef periphery, obtaining sediment cores from all four quadrants (N, E, S, W) surrounding the reef platform.

i) The ROV will be equipped with a CTD to be operational continuously during all dives.

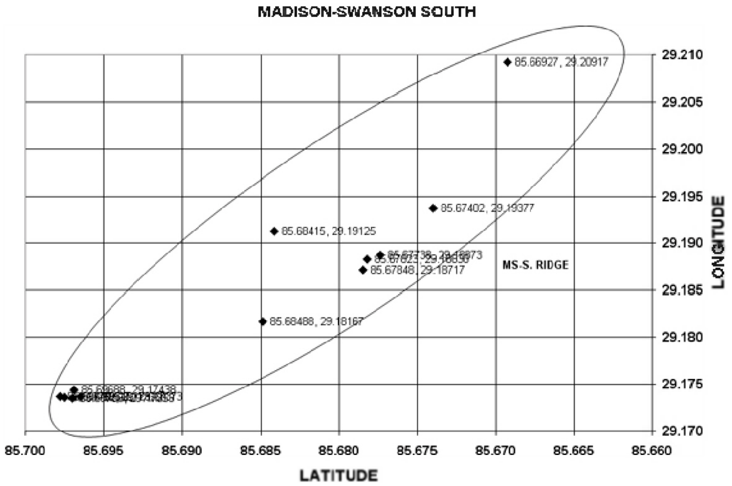
3) Following completion of operations at MSSR, ship will make for the Coral Trees (CT) reference reef site, conducting a series of daytime ROV dives repeating those conducted at CT in 2010.

4) Following completion of CT operations, ship will make for Roughtongue Reef (RTR) and repeat the same sequence of operations as specified above for MSS. Time permitting; 0.5 day of ROV dives will also take place on the closely adjacent Yellowtail Reef (YTR) contingency site. The top of YTR lies about 5 m shallower (65 m) than the top of RTR (70 m). ROV video transecting here would provide a check on potential movement and per unit

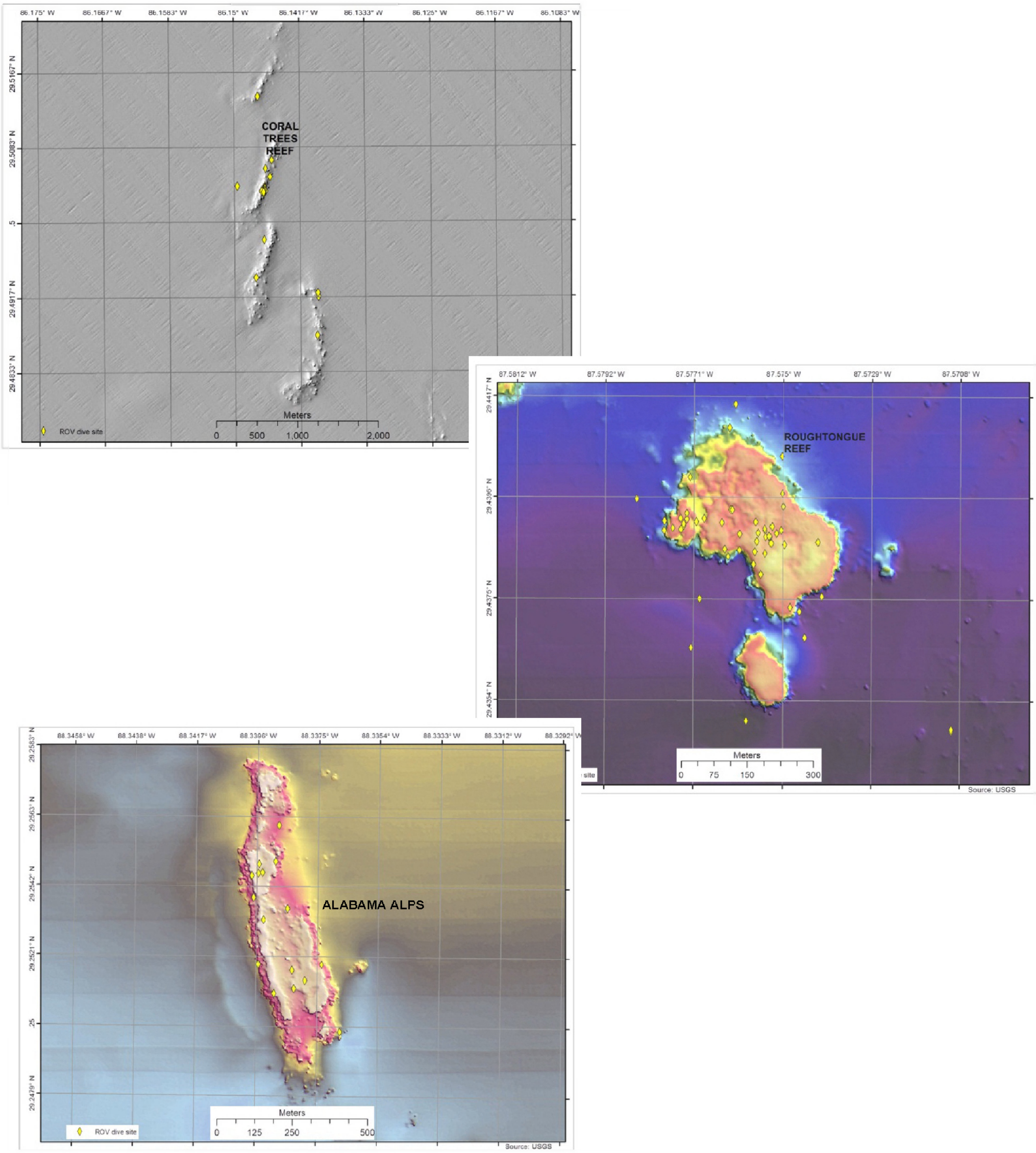
ROV time abundance of fish planktivores (versus RTR) at the shallowest shelf-edge reeftop habitat depths available to these fishes. This may address the question of whether or not low per unit time planktivore abundance observed on RTR in 2010 was a general or depth-specific phenomenon (i.e., planktivore potentially moving to shallower reef depths).

5) Following completion of operations at RTR, ship will make for Alabama Alps Reef (AAR) and repeat the same sequence of operations as specified above for MSS and RTR. It is planned that 0.5 day of ROV dives may take place on the adjacent Talus Block Reef contingency site. The Talus Block Reef lies about 50-90 m deeper (120-160m) than the top of AAR. ROV video transecting here would provide a check on potential movement and per unit ROV time abundance of fish planktivores (versus AAR) at the deepest shelf-edge reef habitat depths available to these fishes. This may also address the question of whether or not low per unit time planktivore abundance observed on RTR in 2010 was a general or depth-specific phenomenon.

APPENDIX D: MESOPHOTIC REEF MISSION, SUPPLEMENTARY SITE MAPS FOR THE PRIMARY TEST AND REFERENCE REEF STUDY SITES.



Diagrammatic zoomed-in latitude-longitude coordinate plot showing all potential MSSR ROV dive locations at the first study reef site to be visited. ROV dives in 2011 will proceed in sequence from SW to NE along the trend of the MSSR reef ridge. During the cruise, similar planning diagrams will be prepared for each study site in advance of operations at each site. Short advance time to schedule cruise deployment precluded preparing all such maps in final form prior to the mission.



Multibeam bathymetry of the CT, RTR, and AAR Study Sites, showing the locations of 2010 NRDA ROV station location, and previous USGS ROV stations (diamonds), providing the basis for planning 2011 NRDA mission ROV station locations and monitoring ROV operations in ArcGis. Full quality maps can be substantial enlarged to track and position ROV 2011 during dives.

APPENDIX E: SAMPLING EQUIPMENT DECONTAMINATION

Decontamination of each core tube will be carried out by washing equipment with soap and water on board between uses. Coring equipment and tubes will be rinsed with fresh water from the vessel, and then rinsed with seawater during descent on the ROV to the sampling site.
Sampling equipment visibly stained with oil or other hydrophobic material will be further decontaminated before use to minimize cross-contamination. While performing the decontamination procedure, phthalate-free gloves, such as nitrile, will be worn. Sampling equipment will be decontaminated in the area designated for decontamination.
The decontamination procedure will proceed as follows:

- Wash and scrub core tubes with detergent.
- Tap water/distilled water rinse.
- An acetone only rinse or a methanol rinse (solvents must be pesticide grade or better) with an optional hexane rinse if necessary after contact by the equipment with visibly contaminated media that prevents complete decontamination at trace levels using the standard procedure.
    - Used solvents will be recovered, stored on board, and disposed of properly when the cruise vessel returns to land.
- Thorough de-ionized (analyte-free) water rinse (if available; otherwise use distilled water).
- Wrap core caps/ends and other sampling equipment that will come into contact with sample matrices in aluminum foil, shiny side out. Remove the aluminum foil before deployment with the ROV.

Sampling equipment being used to collect samples for polycyclic aromatic hydrocarbon (PAH), total extractable hydrocarbon (TEH), or volatile organic carbon (VOC) analyses will utilize the methanol rinse. Solvents used during decontamination activities (e.g., methanol, acetone, hexane) will be collected and handled in accordance with the procedures outlined in the Vessel Safety Plan.

APPENDIX F: MARINE MAMMAL AND SEA TURTLE PROTECTION

Every effort should be made to engage in practices to protect marine mammals and sea turtles. The following forms will be made available to vessel operators and principal investigators on board for dissemination among cruise participants:

- Vessel Strike Avoidance with Ship Strike Form (February 2008 web version),
- NMFS Protocol for Dead Entangled Small Cetaceans,
- Sea Turtle Retrieval Resuscitation Protocols, and
- Turtle Stranding Report Forms.

APPENDIX G: Reporting Guidelines

This Appendix outlines the specific components to be included in the Summary Cruise Report and Final Report for assessment activities conducted under this cooperative work plan.

The Summary Cruise Report will include only the following:

1) General description of activities conducted on the cruise, including locations visited.

2) List of samples collected (e.g., sediments, tissue, whole specimen). Each list should include, where appropriate:
    a. References to date, site, time of collection (latitude/longitude), collection depth, collection method (e.g., grab/suction).
    b. Sample disposition (e.g., intended destination laboratory, archive location).
    c. Intended analysis of sample (e.g., hydrocarbon content, genetic species identification).

3) Identification of data collection issues or deviations from the work plan.

The Final Report will include only the following:

1) Transect Analysis for Fish Abundance:
    a. Transect times used.
    b. Species observed.
    c. Species counts.
    d. Statistical comparison between 2010 and 2011 of fish abundance data.
    e. Statistical comparison between Alabama Alps/Roughtongue Reefs and Coral Trees (2010 data) and Madison Swanson and Coral Trees (2011 data).

3) Rotary Time-lapse Camera Analyses:
    a. Species observed.
    b. Species counts.
    c. Comparison between Alabama Alps/Roughtongue Reefs and Madison Swanson.

4) Coral & Fish Imaging:
    a. Number of corals imaged - referenced by site, time of image collection (estimated latitude/longitude), depth and species/taxa.
    b. Number of fish taxa imaged - referenced by site and time of image collection (latitude/longitude) and depth.
    c. Description of surveyed reef areas, including presence/absence of dead or dying coral:
        i. Quantification methodology.
        ii. Explanation of description/classification system used.
    d. Index of pictures showing representative examples of any notable observed features such as discoloration, sloughing tissue, excessive mucus production, abnormal polyps, abnormal associate occurrence patterns or behavior, and other visible indicators of coral or associated species health status.

5) Water Column Profile Information (CTD Casts):

a. Location (reference latitude/longitude at dive initiation and depth during the dive as referenced by time) and date/time of analytical measurement.

b. Characterization of CTD, turbidity, and fluorometer information.

c. Identification of any observed deviations from background, depressed dissolved oxygen or elevated fluorescence.